

The wheat MYB-related transcription factor TaMYB72 promotes flowering in rice

Summary Through large-scale transformation analyses, TaMYB72 was identified as a flowering time regulator in wheat. TaMYB72 is a MYB family transcription factor localized to the nucleus. Three TaMYB72 homologs, TaMYB72-A, TaMYB72-B and TaMYB72-D, cloned from hexaploid wheat were mapped to the short arm of the group 6 chromosomes. Under the long-day conditions, over-expression of the TaMYB72 in rice shortened the flowering time by approximately 12 d. Expression analyses suggest that TaMYB72 may function through up-regulation of florigen genes *Hd3a* and *RFT1*.

Flowering time in plants is an important trait that determines distribution and regional adaptability, which ultimately affect the seed production of plants (Andres and Coupland 2012). A better understanding of the genetic pathways that control the flowering time of plants may provide effective strategies for engineering high-yield varieties that adapt to different climate conditions and changing environments (Jung and Muller 2009).

Our previous works generated more than 15,000 transgenic rice plants with overexpression of wheat transcription factor genes. One transgenic line, 4L-11-1, which exhibited an earlier flowering phenotype, was identified from the To transgenic plants. Compared with wild-type (WT) plants, 4L-11-1 showed a flowering time shortened by 12.1 d (Figure S1A). Sequence analysis confirmed that the 4L-11-1 transgenic lines contained a transformed wheat gene, TaMYB72. Further evaluation of the segregation of TaMYB72 in T1 plants by polymerase chain reaction (PCR) using primers specific for TaMYB72 indicated that TaMYB72 co-segregated with the phenotype of earlier flowering (Figure S1B; Table S1).

Three TaMYB72 homologs, TaMYB72-A, TaMYB72-B and TaMYB72-D, containing six exons and five introns, were cloned from hexaploid wheat (Figure S2A, B). Through Basic Local Alignment Search Tool (BLAST) analysis of the ordered draft sequence of the hexaploid bread wheat genome that was produced by sequencing isolated chromosome arms (International Wheat Genome Sequencing 2014), the TaMYB72-A, TaMYB72-B and TaMYB72-D genes were assigned to wheat chromosomes 6AS, 6BS and 6DS, respectively (Figure S2C). Using TaMYB72s as the query sequences, 13 homologous MYB protein sequences with unknown function were identified in dicots and monocots. Phylogenetic analysis of TaMYB72s and their homologs was used to generate a phylogenetic tree that contained two main clades. Two barley proteins, BAK02601.1 and J88577.1, shared the closest relationship with the TaMYB72 proteins, and the sequence

identities between these proteins ranged from 94.9% to 97.0%. Other proteins exhibited 56.7% to 83.3% sequence identity with the TaMYB72 proteins (Figure S3). Transient co-expression of the TaMYB72-GFP and nuclear marker OsMADS3-mCherry constructs in rice leaf protoplasts indicated that the TaMYB72 protein is a nuclear protein (Figure S4).

Additional TaMYB72 transgenic lines driven by the maize ubiquitin promoter (*pUbi::TaMYB72*) and the native promoter (*pTaMYB72::TaMYB72*) were produced in the Nipponbare background. Similar to 4L-11-1, flowering of the newly transformed *pUbi::TaMYB72* transgenic plants was accelerated by 12.1 d compared to wild type (WT) plants under natural long day conditions in Beijing, but not under natural short day conditions in Hainan. *pUbi::TaMYB72* transgenic plants also exhibited accelerated flowering under controlled long day conditions (15 h light/9 h dark) but not under controlled short day conditions (9 h light/15 h dark) (Figure 1, S5). Similarly, *pTaMYB72::TaMYB72* transgenic plants exhibited flowering approximately 11.9 d earlier than WT under long day conditions but no change in flowering time under short day conditions (Figure S6). These results demonstrated that TaMYB72 may promote flowering specifically under long day conditions.

To determine whether TaMYB72 also regulates other traits in rice, we investigated the phenotypic data for transgenic rice planted in Beijing and Langfang. Compared with WT, the TaMYB72 transgenic rice lines exhibited reductions in plant height, panicle length, grain numbers and primary branch numbers, while no obvious differences were observed in tiller number or 1000-grain weight between TaMYB72 transgenic rice and WT (Table S2). These results indicate that TaMYB72 may be an important regulator with multiple functions in rice.

To decipher the possible mechanism of TaMYB72 in regulating rice flowering, we compared the expression of several photoperiod-related genes in WT and transgenic plants under long day conditions using quantitative reverse transcription PCR. *Hd3a* and *RFT1*, the two florigen genes, play major roles in the photoperiodic induction of the floral transition in rice (Tamaki et al. 2007; Komiya et al. 2008; Itoh et al. 2010). The expression level of *Hd3a* increased dramatically during light time in TaMYB72 transgenic plants, while the expression of *RFT1* increased at all the time points during the examined 24 h period (Figure 2). Subsequently, the expression of their downstream gene, *OsMADS14*, was induced strongly in the TaMYB72 transgenic plants. *Hd1* and *Ehd1* are two regulators of the expression of *Hd3a* and *RFT1* (Yano et al. 2000; Doi et al. 2004). To determine whether the activation of florigen genes by TaMYB72 is mediated by *Hd1* and/or *Ehd1*, we compared their expression levels in WT and TaMYB72 transgenic plants. The expression of *Ehd1* was induced in the TaMYB72 transgenic plants, while the expression of *Hd1* was not changed in the TaMYB72 transgenic plants,

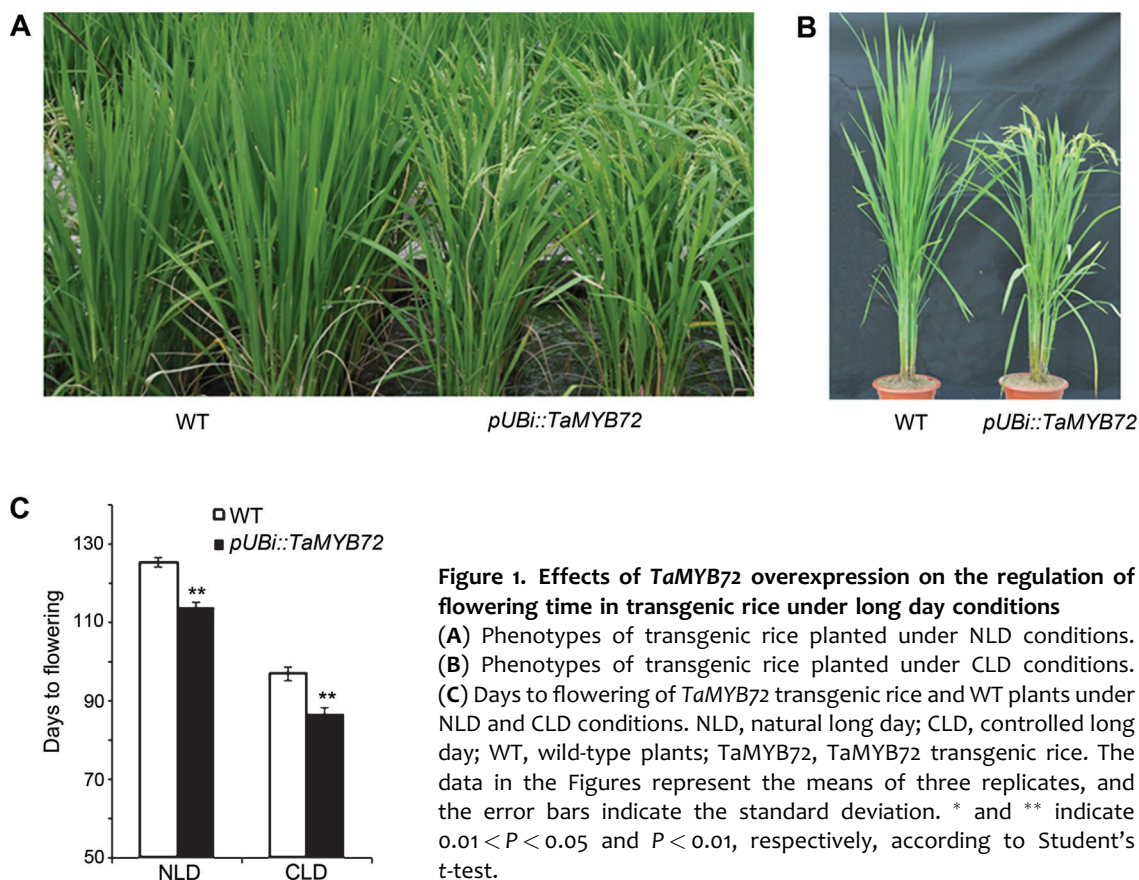


Figure 1. Effects of *TaMYB72* overexpression on the regulation of flowering time in transgenic rice under long day conditions

(A) Phenotypes of transgenic rice planted under NLD conditions. (B) Phenotypes of transgenic rice planted under CLD conditions. (C) Days to flowering of *TaMYB72* transgenic rice and WT plants under NLD and CLD conditions. NLD, natural long day; CLD, controlled long day; WT, wild-type plants; *TaMYB72*, *TaMYB72* transgenic rice. The data in the Figures represent the means of three replicates, and the error bars indicate the standard deviation. * and ** indicate $0.01 < P < 0.05$ and $P < 0.01$, respectively, according to Student's t-test.

indicating that *TaMYB72* plays a role in regulating the flowering mediated by *Ehd1*. We then tested whether *TaMYB72* influences the expression of other known regulators of *Ehd1*, including two positive regulators *OsMADS50* and *OsGI*, and four negative regulators *Se5*, *Ghd7*, *Ghd7.1* and *Ghd8* (Park et al. 1999; Xue et al. 2008; Andres et al. 2009; Yan et al. 2011; Yan et al. 2013). Unexpectedly, the transcription levels of these genes were not significantly affected in the *TaMYB72* transgenic plants. These observations suggest that *TaMYB72* functions upstream of *Ehd1* but independent of the *Ehd1* regulators we examined. Together, these observations suggest that *TaMYB72* may regulate the expression of *Hd3a* and *RFT1* mediated by *Ehd1*, ultimately promoting rice flowering under long day conditions (Figure 2).

In this study, the function of the wheat *TaMYB72* gene was characterized by examining its heterologous expression in rice. These results provide important cues for thoroughly understanding the function and mechanisms of *TaMYB72*.

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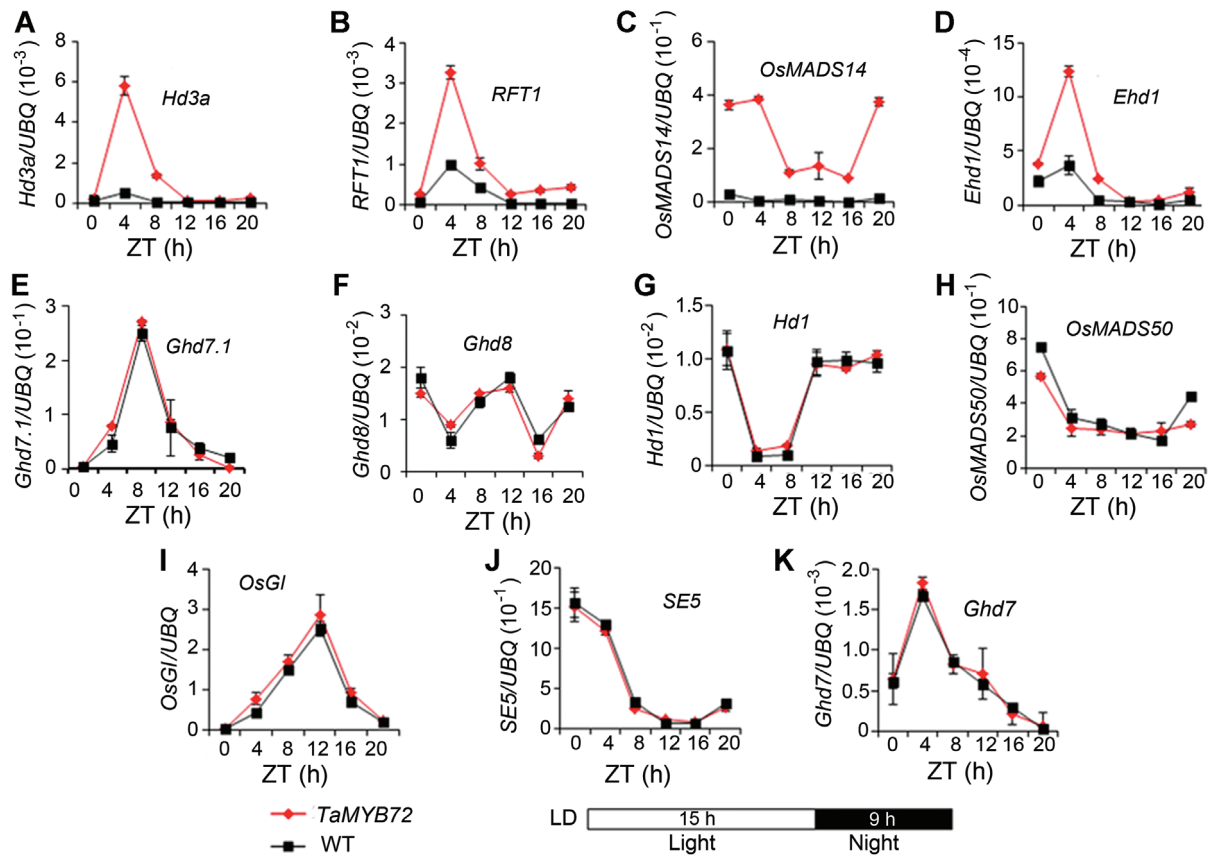


Figure 2. The rhythmic expression patterns of selected genes regulating rice flowering time under long day conditions

The rhythmic expression patterns of *Hd3a* (A), *RFT1* (B), *OsMADS14* (C), *Ehd1* (D), *Ghd7.1* (E), *Ghd8* (F), *Hd1* (G), *OsMADS50* (H), *OsGI* (I), *SE5* (J) and *Ghd7* (K) were examined in *TaMYB72* transgenic and WT plants using quantitative reverse transcription polymerase chain reaction. The open and filled bars at the bottom represent light and dark periods, respectively. The rice *ubiquitin-1* (*UBQ*) gene was used as an internal control. The data in the Figures represent the means of three replicates, and the error bars indicate the SD. WT, wild-type; ZT, zeitgeber time.

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SUPPORTING INFORMATION

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Figure S1. The phenotype of the 4L-11-1 line planted in the field and PCR testing of the T1 plants (A) The phenotype of the 4L-11-1 line in the field. (B) Segregation of *TaMYB72* in T1 plants as determined by PCR using primers specific for *TaMYB72*; 1-20 indicate the different T1 plants of 4L-11-1.

Figure S2. Schematic structures and chromosomal locations of the *TaMYB72* genes (A) Structures of the *TaMYB72* genes. Green rectangles indicate exons, and black lines indicate introns. (B) Structure of the *TaMYB72* protein. The red rectangle indicates the MYB repeat. (C) Chromosomal locations of the *TaMYB72* genes. For the *TaMYB72-A* gene, no PCR-amplified product was generated from the templates lacking chromosome 6A but including NT6A6B and NT6A6D. For *TaMYB72-B*, no PCR-amplified product was generated from the templates lacking chromosome 6B but including NT6B6A and NT6B6D. For *TaMYB72-D*, no PCR-amplified product was generated from the templates lacking chromosome 6D but including NT6D6A and NT6D6B.

Figure S3. Phylogenetic relationships among the *TaMYB72* proteins and their homologs The complete amino acid sequences of the three *TaMYB72* proteins and 13 proteins from other species were aligned using ClustalW and manually adjusted. A neighbor-joining tree was constructed using MEGA 6.0 software with 1000 bootstrap replicates. The

divergence of the clades between the monocots and dicots is indicated by a straight line.

Figure S4. Subcellular localization of the *TaMYB72-B* protein in rice leaf protoplasts (A) The subcellular localization of the *TaMYB72-B*-GFP fusion protein. (B) The nuclear marker MADS3-mCherry fusion protein. (C) Merged images of A and B under brightfield microscopy.

Figure S5. Phenotypes of *TaMYB72* transgenic rice under short day conditions (A) Phenotypes of *TaMYB72* transgenic rice planted in a field in Hainan under NSD conditions. (B) Phenotypes of *TaMYB72* transgenic rice under CSD conditions. (C) Days for flowering of *TaMYB72* transgenic rice and WT plants under NSD and CSD conditions. NSD, natural short-day; CSD, controlled short-day; *TaMYB72*, *pUbi::TaMYB72* transgenic rice; WT, wild-type plants. The data in the figures represent the means of three replicates, and the error bars indicate the SD.

Figure S6. Phenotypes of *pTaMYB72::TaMYB72* transgenic rice (A) Phenotypic comparisons of *pTaMYB72::TaMYB72* transgenic rice, wild type (WT) rice, and *pUbi::TaMYB72* transgenic rice under long day conditions. (B) Days for flowering of the plants in (A). The data in the figures represent the means of three replicates, and the error bars indicate the standard deviation.

Table S1. The segregation by flowering time of 4L-11-1 T1 plants

Table S2. Statistical agronomic trait data for *TaMYB72* transgenic rice lines planted in Beijing and Langfang

Table S3. The sequences of the primers used in this study